2018

Eastern Diamondback Rattlesnake (Crotalus adamanteus) telemetry techniques and translocation

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EASTERN DIAMONDBACK RATTLESNAKE (*CROTALUS ADAMANTEUS*) TELEMETRY TECHNIQUES AND TRANSLOCATION

A thesis submitted to
the Graduate College of
Marshall University
In partial fulfillment of
the requirements for the degree of
Master of Science
In
Biological Sciences: Organismal, Evolutionary
and Ecological Biology
by
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Marshall University
July 2018

APPROVAL OF THESIS

We, the faculty supervising the work of Michael Jungen, affirm that the thesis, *Eastern Diamondback Rattlesnake (Crotalus adamanteus) Telemetry Techniques and Translocation*, meets the high academic standards for original scholarship and creative work established by the Biological Sciences Program and the College of Science. This work also conforms to the editorial standards of our discipline and the Graduate College of Marshall University. With our signatures, we approve the manuscript for publication.

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ACKNOWLEDGMENTS

This thesis is a product of many hours of pain and suffering and certainly wasn't completed alone. First, I would like to thank Dr. Waldron for taking on a northern boy who had never seen a rattlesnake before. Thank you so much for taking that risk. Dr. Waldron and Dr. Welch provided invaluable mentorship that forced me to become a better scientist. Thank you so much to my other advisers Dr. Pauley and Dr. Wiggers in helping make this happen. I couldn't have done this without the constant help and support of the people at Nemours Wildlife Foundation: Ernie Wiggers, Beau Bauer, Justin, Robert, Mr. Ray, and Michelle. John Holloway, Van Horton, and Charles from Parris Island were invaluable in making this all happen. I need to especially thank Zach Ross and Kate Amspacher for helping me do the field work. Herp lab members Jessica Cantrell, Sean Wineland, Rachel Arrick, Maggie Smith, and Emily Maustellar helped keep me sane and provided crucial support when I needed it the most. I would like to extend the most sincere and profound thank you to my parents Mark and Susan Jungen. Without their support and encouragement to follow my dreams I would have never pursued graduate school. I have to thank my best friend Mike Bower. Growing up together, Mike and I nurtured each other's interest and curiosity in this world and implored each other to follow our dreams and seek out a higher understanding. Lastly, thank you to my dog Coosaw for making this experience much harder than it needed to be.

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ABSTRACT

Internal implantation of radio-transmitters is the preferred attachment technique for snakes, but the high costs and invasive nature of the surgery make a functional alternative desirable. External radio-transmitters are cost-effective alternatives to surgical implantation. Rattlesnake rattles are unique morphological features that can serve as an attachment site for external radio-transmitters. Using thread and epoxy, I attached transmitters to the rattles of eastern diamondback rattlesnakes (*Crotalus adamanteus*; EDB). I calculated average monitoring duration using radio telemetry data collected from 49 adult EDBs telemetered from 2014 to 2017 in coastal South Carolina. On average, we monitored EDBs for 189 ±78 days with 14 EDBs monitored > 240 days and 3 EDBs monitored >300 days. External transmitter attachment is a viable alternative to surgical implantation, providing a non-invasive approach to monitoring rattlesnakes. The EDB is a long-lived, large-bodied pit viper endemic to southeastern pine savannas and woodlands. The EDB is declining, and conservation efforts, including long-distance translocation, are being undertaken to aid in the species' recovery. Long-distance translocation to re-establish or supplement populations of viperids has yielded mixed results, with survival averaging less than 50%. I translocated EDBs (N = 21) from a sea island population to a pine savanna restoration area located on private property in South Carolina, 2016-2017, and estimated post-translocation survival probability. I ran various known-fate models in MARK to analyze covariates affecting survival probability. The top model had time since egress as the most important survival covariate, and probability of surviving to the end of the study was 83%. This study will further our understanding of the efficacy of translocation as a conservation tool for EDB restoration.

CHAPTER 1

MONITORING EASTERN DIAMONDBACK RATTLESNAKES USING A NOVEL EXTERNAL RADIO-TRANSMITTER ATTACHMENT METHOD

INTRODUCTION

Prior to the 1990s, studies that focus on snake ecology constituted a small fraction of the ecological literature, but have since increased significantly with the advent of miniature radio-transmitters (Shine and Bonnet, 2000; Beaman and Hayes, 2008; Dorcas and Willson, 2009). Miniature radio-transmitters allowed researchers to experiment with a variety of attachment techniques, including force-feeding (Osgood, 1970; Fitch and Sheier, 1971; Jacob and Painter, 1980; Shine and Lambeck, 1985; Rivas, 2001), external adhesion (Gent and Spellerberg, 1993; Cobb et al., 2005; Jellen and Kowalski, 2007; Tozetti and Martins, 2007; Figueroa et al., 2008; Madrid-Sotelo and García-Aguayo, 2008; Wylie et al., 2011; Howze et al., 2012; Riley et al., 2017; Robinson et al., 2018), subcutaneous attachment (Ciofi and Chelazzi, 1991; Riley et al., 2017), and intracoelomic (surgical) implantation (Reinert and Cundall, 1982; Madsen, 1984; Weatherhead and Anderka, 1984; Cobb et al., 2005; Lentini et al., 2011). While each attachment technique has its own set of advantages and disadvantages, surgical implantation is the most popular and frequently used technique for radio-transmitter attachment (Reinert, 1992; Dorcas and Willson, 2009; Cardwell, 2017).

Surgical transmitter implantation is popular, in part, because it allows for long monitoring duration (e.g., two years), has a low risk of detachment, and desirable safety record (Reinert, 1992; Dorcas and Willson, 2009; Cardwell, 2017). Other methods of attachment, such as glue-on or tape-on techniques, can detach prematurely and can cause skin irritation, injury, scarring, and/or death (Ujvari and Korsos, 2000; Jellen and Kowalski, 2007; Tozetti and Martins, 2007;

Wylie et al., 2011; Riley et al., 2017). Force-feeding is seldom used as it has a short monitoring duration and affects snake movement and thermoregulation (Lutterschmidt and Reinert, 1990; Reinert, 1992). Some external transmitter attachment techniques (e.g., subcutaneous placement and taping/gluing) have shown promise as cost-effective alternatives to surgical implantation, but they still fall short in terms of reliable attachment and monitoring duration as well as animal health in some cases (Cioffi and Chelazzi, 1991; Jellen and Kowalski, 2007; Tozetti and Martins, 2007; Figueroa et al., 2008; Riley et al., 2017).

Given logistical constraints of conducting sterile surgery in the field, most studies that use surgical implantation require access to sterile/clean facilities (or access to a trusted veterinarian), which may not be applicable for remote study sites (Anderson and Talcott, 2006; Tozetti and Martins, 2007). Furthermore, veterinarian costs can strain budgets given that at least two surgeries are required per snake (i.e., implantation and removal) (Goodman et al., 2009; Robinson et al., 2018). Transmitter implantation surgery requires time to recover from the incision, altering behavior in the short term (e.g., sedentariness, basking, fasting, and ecdysis) (Rudolph et al., 1998; Weatherhead and Blouin-Demers, 2004; Lentini, 2008; Wylie et al., 2011). The antenna of the transmitter can protrude from the body or wrap around organs (Hardy and Greene, 1999; pers. obs.) and abscesses can form around or near the transmitter (Lentini et al., 2011; pers. obs). Additionally, snakes can get infections from surgery and even die (Rudolph et al., 1998; Lentini et al., 2011). Finally, a surgically implanted transmitter that dies prematurely could have unknown adverse effects for the snake if it is not recovered and the transmitter removed (Wylie et al., 2011). While these problems occur rarely, there is no doubt researchers would avoid them if possible.

Rattlesnakes (genus *Crotalus*) have been ideal models for snake telemetry studies since their large body size allows researchers to attach large transmitters and track them long enough to answer many research questions (Ujvari and Korsos, 2000). Elevated risk perceptions and negative attitudes toward rattlesnakes provides a basis for monitoring their movements and behavior in areas of co-occurrence with humans (Gibbons and Dorcas, 2002; Waldron et al., 2013b). Attitudes toward rattlesnakes are changing and many people who find them on their property would prefer to have them moved instead of killed (Nowak et al., 2002). These nuisance rattlesnakes provide researchers with the opportunities to study the effects of moving these snakes using telemetry.

Here, I examine the utility of external transmitter attachment on the rattlesnake rattle as an alternative means of radio telemetrically monitoring free ranging rattlesnakes in long-term studies. I expected that rattle-anatomy provided a unique transmitter attachment location that would pose little threat to survival and minimally affect behavior. Unlike other external transmitter attachment methods, a rattle attachment approach limits transmitter contact with skin (i.e., reducing risk of skin lesions), and would not be detached when rattlesnakes shed, which means attachment to the rattle could serve as a long-term monitoring technique since shedding is a leading cause of losing study snakes (Riley et al., 2017).

Starting in 2011, Dr. Jayme Waldron began externally attaching radio-transmitters to rattles of eastern diamondback rattlesnakes (*Crotalus adamanteus*; EDBs). Initially, the goal was to attach transmitters to EDB rattles as a means to temporarily monitor snakes (e.g., pregnant females and overwintering snakes that were captured outside of the surgery window). For example, she attached external transmitters to snakes that had internal transmitters with batteries that would expire while the snakes overwintered underground, ensuring the retention of study animals (i.e., to avoid late season implantation, Rudolph et al., 1998) until it could be taken to surgery in the Spring. Here, I present the methodology for attaching radio-transmitters to EDB rattles as a reliable, long-term, non-invasive, cost-effective alternative to surgical implantation for monitoring large-bodied, free-ranging rattlesnakes.

METHODS

I captured EDBs on the Marine Corps Recruit Depot (MCRD), Parris Island, South Carolina, USA, using visual surveys in habitat, incidentally on roads, and during radio-telemetry monitoring efforts. After capture, I processed rattlesnakes using snake hooks and clear restraining tubes to measure snout-vent-length (SVL; cm), mass (g), total length (TL; cm), to mark using PIT tags, and to attach the radio-transmitter (Model R1640; Advanced Telemetry Systems, Isanti, MN, USA; 2g, 9-11 by 5 by 22mm; Pulse rate: 17ppm, Pulse width: 15ms; battery life: 240 days) to the rattle. The radio-transmitter was less than 1% of the total body mass and had a maximum width ≤ the width of the rattle, and thus conducive for attachment to the rattle without hindering movement or behavior.

I attached radio-transmitters to the lateral surface of the rattle using quilting thread and epoxy (Figure 1). I tied the transmitter to the rattle by wrapping the thread between each rattle segment along the length of the transmitter. Starting at the base of the transmitter, leaving extra thread for tying a knot, I wrapped the thread around the transmitter and the space between rattle segments four times and then proceeded to the next rattle segment division. I wrapped the thread around the transmitter and the space between rattle segments four times for each additional segment division along the length of the transmitter. I wrapped thread around the transmitter and the space between rattle segments eight times at the distal end of the transmitter, then wrapped thread around the transmitter and the space between rattle segments four additional times moving back toward the proximal end of the transmitter before tying a knot at the base of the transmitter, such that each rattle division was wrapped with thread eight times before the knot was tied. I did not standardize knot placement because I completely covered the knot with a one minute setting epoxy, eliminating the risk of coming untied. I applied epoxy to the transmitter and adjacent rattle segments. I covered the entire attachment area with epoxy including the thread between rattle segments, the area where the distal end of the transmitter meets the rattle, and the area where the base of the transmitter meets the first rattle segment. I allowed the epoxy to dry completely while the snake was restrained to ensure that the transmitter did not adhere to the snake's skin or the holding container. Following processing, the snake was released at its capture location. I used the external attachment technique on snakes with a range of rattle segments (0-13) (Figure 2).

Figure 1. Radio-transmitter attached to rattle with thread and epoxy.

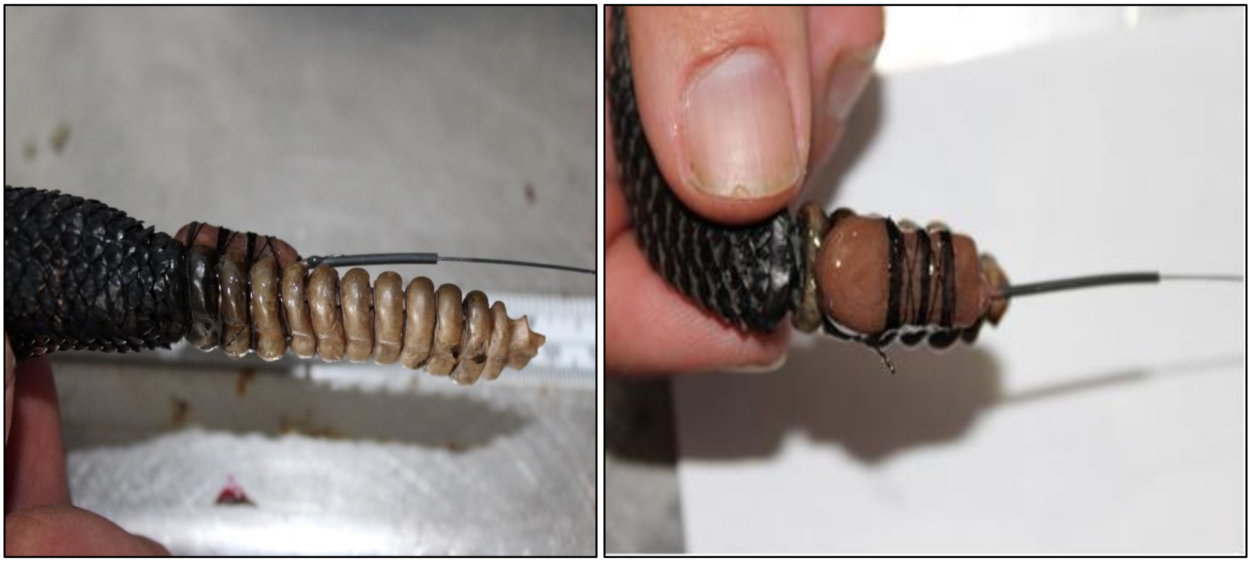

Figure 2. Rattle attachment examples. Rattle with radio-transmitter attached to the basal rattle with many segments (left) and shorter rattle with radio-transmitter attached a couple segments above the basal rattle (right).

Some snakes used in this analysis had internal transmitters. Intent of all transmitter attachments fell into two categories: transmitter attachment as a temporary measure until I could bring snakes to surgery (temporary) and transmitter attachment to track the snake for the entire battery life of the transmitter (long-term). I used descriptive statistics to evaluate the effectiveness of the attachment technique for monitoring adult EDBs. I calculated total monitoring duration in days for each individual and calculated mean monitoring duration and percent battery life used for all individuals. Monitoring duration ended one of two ways: the transmitter detached and the snake was lost (dropped) or the transmitter did not detach and the snake was captured to replace or remove the transmitter (retained). Four snakes were intentionally killed by humans after 81-135 days and were removed from analyses because tracking duration did not reflect the utility of the attachment technique. I conducted multiple analyses using all categories of monitored snakes: temporary, long-term, dropped, and retained. I analyzed all snakes (n = 49) regardless of outcome or intent, then analyzed the data based on outcome (dropped vs retained; n = 49) and intent (temporary vs long-term; n = 49) to better understand the utility of the attachment technique. I used correlation analysis in SAS 9.4 to examine the relationship between the number of rattle segments at the time of transmitter attachment and the duration an individual was tracked. I ran a t-test to examine the difference in monitoring duration between dropped and retained EDBs. The purpose was to see if the number of rattle segments affected the attachment duration.

RESULTS

I attached external radio-transmitters to 52 adults and one juvenile and monitored them from September 2014 to October 2017. Transmitter batteries did not expire prematurely in this study. No snakes were injured or died as a result of attaching a radio-transmitter to their rattle. One post-partum female was depredated during the course of this study but was likely left vulnerable from giving birth the previous Fall rather than affected by an attached external transmitter. Total monitoring duration varied across individuals (range: 28 – 361 days). Mean monitoring duration for all snakes with external radio-transmitters attached to their rattle was 189 days (±79), or 79% of transmitter battery life (Table 1). Snakes that dropped their transmitter (n = 22) were monitored for 156 days, on average (±77). Snakes dropped their transmitters when the transmitter got caught in thick vegetation and pulled the rattle off, in most cases. Snakes that did retain their transmitters (n = 27) were monitored 205 days, on average (±70) (Table 1). Transmitters attached with an intention to monitor for the entire battery life (n = 33) were retained for 205 days, on average (±88). Temporarily attached transmitters were retained for 155 days, on average (±40) (Table 1). I monitored 14 snakes for more than 240 days. I monitored three snakes for more than 300 days and one snake for 361 days, which is 60 and 121 days longer than the guaranteed battery life respectively. Conversely, I monitored ten snakes for less than 120 days and two snakes for less than 60 days, although three of these snakes were monitored temporarily before being captured and taken to surgery.

Number of rattle segments was not correlated with monitoring duration ($r_{49} = 0.04$, $p = 0.80$). Average number of rattle segments at the time of transmitter attachment was 6 segments (±3). The number of rattle segments ranged from zero (i.e., the basal/blood rattle) to 13 segments. Removing and/or replacing radio-transmitters attached to the rattle did not damage rattles. Dropped EDBs had a significantly smaller monitoring duration than retained EDBs ($t_{47} = 2.78$, $p < 0.008$). All EDBs with transmitters attached could still rattle, although with some muffling.

Table 1. Average monitoring duration with standard deviation in days of all snakes. Average monitoring duration of all snakes calculated by outcome (dropped or retained) and intent (long-term or temporary). Percent battery life is the average percent of total battery life (240 days) the snake was monitored.

Category	N	Duration (days) [σ]	Battery Life (%)
All	49	189 [79]	79
Outcome:			
Dropped	22	156 [77]	65
Retained	27	216 [70]	90
Intent:			
Long-term	33	205 [88]	86
Temporary	16	155 [40]	64

DISCUSSION

Attaching radio-transmitters to EDB rattles has shown great potential as a noninvasive, functional transmitter attachment method with long-term tracking capabilities. The long-term category of EDBs is most representative of a study using only transmitters attached to rattles. Long-term EDBs were tracked for over six months, on average, which is a significant increase compared to other external transmitter attachment options. I saw no difference in the effectiveness of this technique based on the number of rattle segments an EDB had at transmitter attachment. No EDBs were injured or died from rattle transmitter attachment.

As with many other external transmitter attachment methods, rattle attachments are susceptible to detaching from the snake. Transmitter detachments usually result from the rattle being pulled off by either vegetation or other structures. I lost 22 of 47 snakes in this study from rattle detachment. I suspect that transmitters are more likely to detach after subsequent sheds because the transmitter moves further away from the basal rattle. Most transmitters detached in tight spaces (e.g., small, tight root holes and stump holes) and in dense vegetation (e.g., thick patches of yaupon holly). Despite losing 22 EDBs to transmitter detachment, I still monitored these individuals for 156 days, on average. For comparison, other studies using external transmitters on other rattlesnakes had monitoring durations ranging from 39-76 days (Cobb et al., 2005; Jellen and Kowalski, 2007; Tozetti and Martins, 2007; Figueroa et al., 2008; Howze et al., 2012; Riley et al., 2017).

Rattle transmitter attachment provides an alternative, noninvasive attachment method for rattlesnake telemetric studies. This method allows studies to have much larger sample sizes, since veterinarians are not needed and transmitters are cheaper. Rattle transmitter attachment has no physical costs to the study organism and impacts behavior no more than initial handling and measuring. This technique does not silence the rattle and transmitters can be replaced with ease. This method is versatile, can be attached *in situ*, and can be used for both long- and short-term studies. I especially suggest the rattle attachment method for tracking gravid females. Conducting surgery on gravid females, especially females of large, long-lived species, can add extra stress to an exceedingly stressful life history constraint. For example, adult EDBs do not reach sexual maturity until ~7 years (Waldron et al., 2013a). Once sexually mature, females only breed once every 2-4 years (Timmerman and Martin, 2003). A gravid female will emerge at egress and not feed until after parturition in August, at which time she needs a meal before hibernation (Wallace and Diller, 1990; Rubio, 2010). An alternative transmitter attachment method is needed for gravid females since we do not want to lower reproductive success of a species that breeds only once every 2-4 years and could potentially die after parturition if she does not reach a healthy body condition to withstand hibernation. Gravid females have been found to reabsorb follicles after surgical implantation of radio-transmitters, which may have caused a depletion of energy reserves required for reproduction (Graves and Duvall, 1993).

Rattle transmitter attachment works on other rattlesnake species. I have tracked timber rattlesnakes (*Crotalus horridus*) on properties in the coastal plains using rattle transmitter attachment with similar results. I believe this method could be functional for many different species of rattlesnakes. Rattlesnake species in higher latitudes, with shorter growing seasons could benefit from our transmitter attachment method. At northern latitudes, where the active season is short, surgical implantation would be more invasive, where the recovery time would take away from crucial foraging opportunities. Snakes would need to commit more time to recovery from surgery, which would take away from foraging and possibly reproduction.

The ease of attachment, cost-effectiveness, reliability, and long-term monitoring capabilities of this external transmitter attachment method has a wide scope of research functionality. I expect the method provides researchers with a versatile tool to monitor rattlesnake-human interactions (i.e., nuisance rattlesnakes) as well as shed light into some understudied areas of rattlesnake ecology (e.g., juvenile behavior and reproduction) especially as such research continues its upward trend. Despite the success using rattle transmitter attachment, I expect that surgical implantation is more appropriate for studies of rare species and those that occur in low densities.

CHAPTER 2

LONG-DISTANCE TRANSLOCATION OF EASTERN DIAMONDBACK RATTLESNAKES (*CROTALUS ADAMANTEUS*)

INTRODUCTION

Habitat destruction and fragmentation are the greatest threats to wildlife species (Fahrig, 1997; Spear et al., 2017). Without suitable and connected habitats, populations become isolated and, as available habitat shrinks, meta-populations suffer from an inbreeding depression (Andrén, 1994; Madsen et al., 1996; Frankham et al., 2002; Spears et al., 2017). Many permanent barriers to dispersal and gene exchange exist for many wildlife populations (Eigenbrod et al., 2008), thus, conservationists need to use other management tools, such as translocation, to allow these species to repatriate, colonize, and reestablish populations in areas of suitable habitat within the historic distribution (Griffith et al., 1989; Madsen et al., 1999).

Long-distance translocation (LDT), i.e., translocation to an area outside of an organism's home range (Hardy et al., 2001), is an approach to move and repatriate populations that do not readily disperse (Griffith et al., 1989; Dodd and Seigel, 1991; Macmillan, 1995; Fischer and Lindenmeyer, 2000). Species are typically translocated to areas within their historic distribution, and translocation success varies by taxa (Griffith et al., 1989; Dodd and Seigel, 1991; Reinert, 1991). Snakes, and herpetofauna in general, have low survival when translocated to new landscapes (Burke, 1991; Dodd and Seigel, 1991). Herpetofauna are often poor dispersers and are vulnerable to habitat fragmentation and destruction (Gibbons et al., 2000). Snake LDT faces further obstacles because of lack of protection, ophidophobia, and public distain or misunderstanding (Reinert, 1991; McCrystal and Ivanyi, 2008). Also, snake translocations are not necessarily done for conservation, as mitigation translocations are becoming more popular (McCrystal and Ivanyi, 2008; Massei et al., 2010; Miller et al., 2014).

Venomous snakes are frequently translocated as a result of someone finding the snake in their yard or in public areas (Sealy, 1997; Reinert and Rupert, 1999; Hardy et al., 2001; Nowak et al., 2002; McCrystal and Ivanyi, 2008). However, a growing body of literature indicates many negative repercussions of translocating snakes both inside (short-distance translocation; SDT) and outside (LDT) of their home range (Hare and McNally, 1997; Sealy, 1997; Reinert and Rupert, 1999). Snakes have excellent spatial awareness and exhibit homing behavior (Germano and Bishop, 2009). The increased movements associated with translocation results in high metabolic costs, aberrant movements, vulnerability associated with risky movements (e.g., crossing roads), increased vulnerability to predation, a greater likelihood of encountering humans, and death (Hare and McNally, 1997; Bonnet et al., 1999; Reinert and Rupert, 1999; Plummer and Mills, 2000; Hardy et al., 2001; Nowak et al., 2002; Butler et al., 2005). Wildlife officials also use LDT and SDT to deal with nuisance snakes with similar results (Devan-Song et al., 2016). The difference being LDT typically results in the nuisance snake not returning to the capture location (Reinert and Rupert, 1999; Hardy et al., 2001).

Venomous snake translocation is often performed under the context of conflict mitigation, although the effectiveness of LDT is poorly understood (Miller et al., 2014; Germano et al., 2015). Low survival post-LDT is driving recommendations against using LDT for conservation and conflict mitigation (Reinert and Rupert, 1999; Plummer and Mills, 2000; Hardy et al., 2001; Nowak et al., 2002; Butler et al., 2005; Devan-Song et al., 2016). Despite these problems, LDT may be the only option for conserving species that cannot colonize or re-establish populations naturally (Tuberville et al., 2005). In addition, many different aspects of translocation such as phenology, habitat integrity/suitability, and movement ecology have been acknowledged as factors contributing to LDT success but are understudied (Griffith et al., 1989; Dodd and Seigel, 1991; Plummer and Mills, 2000; King et al., 2004; Germano and Bishop, 2009).

Phenology is largely ignored in venomous snake LDTs with most translocations occurring at the point of encounter or when it is easiest to catch the snakes (e.g., egress). Captive eastern massasaugas (*Sistrurus catenatus*) had higher survival when released during summer as compared to those released in autumn (King et al., 2004). Bright and Morris (1994) found evidence of a seasonal effect of translocation on a mammal species, and it follows that other taxa may also show similar seasonal effects of translocation. Catching and moving venomous snakes may be easiest at egress, but egress may not be the most appropriate time of year for LDT since most snakes do not eat during the inactive season and are vulnerable, exhibiting poor body condition at emergence (Wallace and Diller, 2001; Waldron et al., 2013a). Allowing venomous snakes to egress and spend time foraging before LDT could improve survival post-translocation.

Using source populations that have small home ranges, such as island populations and populations constricted by anthropogenic activity, could be another factor to consider for improving LDT as a conservation tool. Venomous snakes with small home ranges may be more sedentary post-translocation, which would mean less metabolic costs and fewer encounters with predators. Finally, considering habitat quality and management of recipient site as it pertains to the study species' historic landscape is an important predictor of a successful translocation (Griffith et al., 1989; Dodd and Seigel, 1991; Germano and Bishop, 2009).

In this study, I examined the utility of LDT for managing EDB populations. Specifically, I moved/translocated two EDB cohorts, one that was moved at egress and the other that was moved in the active season, allowing me to examine post-translocation survival as a function of phenology. I expected phenology to influence survival post-LDT and, specifically, I expected EDBs that were moved during the active season to exhibit higher survival as compared to EDBs that were moved during egress. Finally, I expected post-LDT home ranges to be much larger than pre-LDT home ranges. The success of this LDT study could shed light on the importance of phenology and the characteristics of both the source population and the recipient site. This study could further our understanding of particular aspects affecting LDT success or failure and guide future rattlesnake LDT conservation efforts.

METHODS

Study Species

The EDB is endemic to the southeastern Coastal Plain and is the largest rattlesnake in North America (Ditmars, 1936; Klauber, 1956). Eastern diamondbacks exhibit a slow life history characterized by delayed maturation (~7 years), low fecundity, and high longevity (>30 years) (Waldron et al., 2008; Waldron et al., 2013a). The eastern diamondback is in decline across its historic range and is a candidate species for protection under the Endangered Species Act (Martin and Means, 2000; U.S. Department of the Interior, 2012). Declines have been linked to habitat destruction, habitat fragmentation, and human persecution (Gibbons et al., 2000; Martin and Means, 2000; Timmerman and Martin, 2003; Means, 2009). Recently, EDBs have been identified as a species of global conservation priority because of its ecological and evolutionary distinctiveness (Maritz et al., 2016). Eastern diamondback conservation is complicated by high site fidelity and specificity to pine savanna woodland habitat (Timmerman and Martin, 2003; Waldron et al., 2008; Hoss et al., 2010; Waldron et al., 2013a). Habitat destruction and fragmentation are the biggest threats to EDB populations since adult EDBs are unlikely to disperse at the landscape scale and neonate survival is low (Waldron et al., 2006; Waldron et al., 2013a). Limited dispersal, combined with the patchy distribution of suitable EDB habitats, make it unlikely that EDBs are able to colonize isolated habitats that have been restored to pine savanna woodland structure (Waldron et al., 2013a).

Study Sites

The Marine Corps Recruit Depot (MCRD), Parris Island is a sea island in Beaufort County, South Carolina and the donor population for translocated EDBs. Parris Island is 3,256 ha of dry land, tidal marsh, and creeks with extensive infrastructure for training, military housing, and a golf course. Training fields containing various training structures and obstacles along with necessary maintenance and operational structures occupy much of the island. Administrative and personnel buildings, as well as a golf course, occupy the other anthropogenic portions of the island. The remaining areas include maritime forests and planted pine (species) managed for wildlife and timber production. Parris Island has a sizable, healthy EDB population that we have been monitoring since 2008 as part of a long-term mark-recapture study. We selected individual EDBs for translocation based on three criteria: human encounter history, proximity to training or residential areas, and likelihood of human conflict.

Nemours Wildlife Foundation (Nemours) in the ACE (Ashepoo, Combahee, and Edisto rivers) Basin is a private, nonprofit organization in northern Beaufort County, South Carolina that was used as the recipient site for translocated EDBs. Nemours (4,000 ha) consisted of diverse habitats, including fresh and brackish marsh, remnant rice field and impoundments, upland pine savanna, hardwood bottom forest, and cypress/tupelo forests. Habitats were maintained and enhanced according to the foundation’s mission to develop and use management practices that conserve and sustain wildlife populations and their habitats. Nemours manages a

280-ha pine savanna restoration area characterized by low basal area, mature pines, and open canopy managed with intensive prescribed burning, herbicide application, and thinning. The pine savanna restoration area was used as the release site for translocated EDBs and had restricted access and minimal human activity. Historically, Nemours supported EDBs, although the species had not been detected at the site since 2012. Nemours served as the recipient location for EDBs that were translocated approximately 32 km from the MCRD with the goal of re-establishing a breeding population of EDBs.

Radio-telemetry and Translocation

I captured EDBs on Marine Corps Recruit Depot (MCRD), Parris Island, South Carolina, USA, from January 2015 to March 2017 using visual surveys in habitat, incidentally on roads, and during radio-telemetry monitoring efforts. After capture, I processed rattlesnakes using snake hooks and clear restraining tubes to measure snout-vent-length (SVL; cm), mass (g), total length (TL; cm), and mark using PIT tags. I brought each EDB to a veterinarian to surgically implant a radio-transmitter (SI-2, 11-13 g, Holohil Systems, Carp. ON) following procedures modified from Reinert and Cundall (1982). Over the course of the study, I attached external radio-transmitters (Model R1640; Advanced Telemetry Systems, Isanti, MN, USA; 2g, 9-11 by 5 by 22 mm; Pulse rate: 17 ppm, Pulse width: 15 ms; battery life: 240 days) to the rattle of some EDBs as needed (Jungen et al., in prep).

I translocated EDBs from Parris Island, SC to Nemours Wildlife Foundation from March 2016 to August 2017. I translocated a cohort of 10 EDBs in March/April of 2016 (spring 2016) and a cohort of 11 EDBs in July/August of 2017 (summer 2017). I released EDBs in the pine savanna restoration area at Nemours, choosing specific drop off sites with plenty of cover and suitable hibernacula. I used radio-telemetry to monitor movements and survival post-translocation. I tracked each EDB for four consecutive days after release to ensure I did not lose individuals that attempted to leave the study area. I located individuals once every two-three days during the active period (mid-March to early November) and once weekly during the inactive period (November to early March).

Statistical Analyses

I calculated survival estimates using radio-telemetry data for known-fate models in program MARK (White and Burnham, 1999; Waldron et al., 2013a). I modeled weekly survival for the first 39 weeks post-translocation since 39 weeks was the shortest amount of time an individual was tracked after release on Nemours. I formatted the encounter history to start on the release date for each EDB and end 39 weeks later. Thus, the encounter history file contained 39 weekly live/dead entries. I ran six candidate models which included survival as a constant, and as a function of SVL (SVL), body condition (BC), sex, average daily movements post-translocation (ADM), and time since egress (TSE). I z-transformed SVL, ADM, and TSE. I did not use cohort as a covariate since it was highly correlated with TSE. I recorded SVL and mass of each EDB at the time of capture for translocation to calculate BC at the time of release. I calculated BC using Fulton's index, which is mass divided by cubed length (Peig and Green, 2010). I chose March 15th as the egress date for calculating TSE. I counted the number of days since March 15th that I translocated each EDB to determine TSE. I used the as.traj() function from the adehabitatLT package in program R to calculate the distance traveled (m) for each EDB during the active season (Calenge, 2006; R Development Core Team, 2018). I divided distance traveled (m) by the number of days post-translocation until the beginning of ingress in mid-late November to calculate average daily movements post-translocation. I compared candidate models using Akaike's Information Criterion for small sample size (AIC_c). Candidate models with $\Delta AIC_c \leq$

2.00 were supported models. I calculated 90% confidence intervals of beta estimates to evaluate each covariate's effect.

I ran a separate survival analysis for Parris Island (i.e., the source population) EDBs that were not translocated for both 2016 and 2017 to examine a year effect on survival since TSE and cohort (i.e., year) are correlated. I calculated survival estimates using radio-telemetry data for known-fate models in program MARK. I modeled weekly survival for the same 39 weeks as the post-translocation survival analysis. I formatted the encounter history to start at egress of that year (2016 or 2017) for each EDB and end 39 weeks later. Thus, the encounter history file contained 39 weekly live/dead entries. The one candidate model was survival as a function of year (i.e., 2016 or 2017).

As a post hoc analysis, I used two t-tests in SAS to examine differences in BC at the time of translocation between cohorts and differences in average daily movements between cohorts. I excluded the one gravid female from the ADM comparison because gravid females are more sedentary than non-gravid females. I used the adehabitatHR package in program R to calculate 85% minimum convex polygons (MCP) for home ranges of each translocated EDB both pre and post-translocation (Calenge, 2006; R Development Core Team, 2018). I chose 85% MCPs for home ranges in order to exclude unused areas from home ranges (e.g., ponds). I ran a paired t-test in SAS to examine differences in home-range size before and after translocation. I excluded two males from analysis because they died less than a month post-translocation.

RESULTS

I monitored 20 (10 males 10; 10 females 10) of the 21 translocated EDBs over 39 weeks, i.e., one female was released and never found again possibly due to transmitter failure. Three (2 males and 1 female) EDBs from spring 2016 cohort and one female from summer 2017 cohort died over the course of the study. Causes of death were predation (n = 1), fecal compaction (n = 2), and broken spine (n = 1). One gravid female from Spring 2016 gave birth to at least ten neonates, which were captured, marked, and released. I observed pairing (n = 10), courting (n = 1), and copulation (n = 1) during the breeding seasons in both cohorts.

The probability of surviving to the end of the study was 83% (±10%). Two candidate models received support with a $\Delta AIC_c \leq 2.0$ (Table 2). The top model included survival as a function of TSE. Time since egress was positively associated with survival ($\beta = 0.94 \pm 0.64$, 90% CI: -0.09 to 1.97); however, our confidence intervals included zero. The constant survival model was also supported ($\beta = 5.11 \pm 0.50$, 90% CI: 4.29 to 5.93).

Table 2. Known-fate survival models ranked in order of support post-translocation. Models with ΔAICc values below 2.0 show support. TSEz = time since egress (days), ADMz is average daily movements (m/day), BC is body condition (Mass/Length3), and SVLz is snout-vent length (cm).

Model	AIC_c	ΔAIC_c	AIC_c Weight	Likelihood	Parameters
S(TSEz)	50.18	0.00	0.354	1.00	2
S(.)	50.93	0.75	0.243	0.69	1
S(ADMz)	52.20	2.03	0.128	0.36	2
S(BCz)	52.80	2.62	0.095	0.27	2
S(SVLz)	52.93	2.75	0.089	0.25	2
S(Sex)	52.93	2.75	0.089	0.25	2

Four EDBs (2 males and 2 females) from 2016 and two EDBs (male and female) from 2017 died over the course of the study on Parris Island. Causes of death were road casualty (n = 1) and human encounter (n = 5). The probability of surviving over the two year period was 83% (±6%). One candidate model received support with a ΔAIC_c value below 2.0 (Table 3). The top model was the constant survival model ($\beta = 5.37 \pm 0.40$, 90% CI: 4.71 to 6.03). Survival had a negligible relationship with year ($\beta = -0.003 \pm 0.86$, 90% CI: -1.42 to 1.41).

Table 3. Known-fate survival models ranked in order of support for Parris Island EDBs. Models with ΔAICc values below 2.0 show support. S(.) is constant survival and S(Year) is survival as a function of year.

Model	AIC_c	ΔAIC_c	AIC_c Weight	Likelihood	Parameters
S(.)	78.48	0.00	0.732	1.00	1
S(Year)	80.48	2.01	0.268	0.37	2

On average, EDBs moved 24 (±7) meters/day and 27 (±14) meters/day post-translocation for spring 2016 and summer 2017 cohorts, respectively. Average daily movement for all translocated EDBs was 25 (±11) meters/day (Figure 3). There was no difference between ADM post-translocation between spring 2016 and summer 2017 cohorts ($t_{18} = -0.5$, $p = 0.62$). The spring 2016 cohort had a significantly better BC at the time of translocation as compared to the summer 2017 cohort ($t_{17} = 2.29$, $p = 0.03$). On average, EDB pre-translocation home range was 9.9 (±14.5) ha and post translocation home range was 16.5 (±14.8) ha. There was no difference between pre and post-translocation home range size ($t_{17} = -2$, $p = 0.06$).

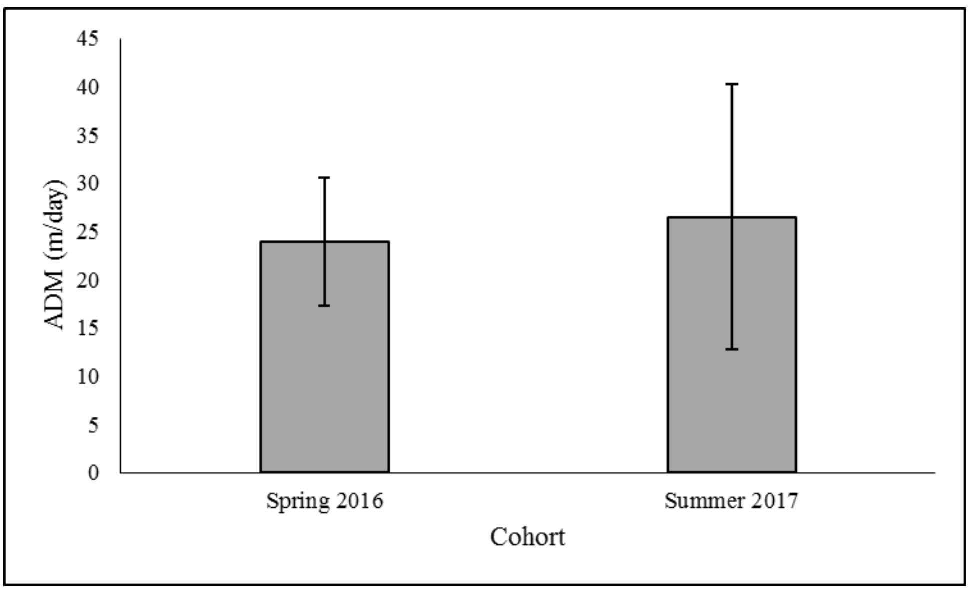

Figure 3. Average daily movements post-translocation of spring and summer EDB cohorts.

DISCUSSION

Long-distance translocation appears to be a viable conservation tool to mitigate EDB imperilment. The overall probability of EDBs surviving until the end of the study was high for a venomous snake LDT study. The 2016 and 2017 survival probability for non-translocated EDBs on Parris Island was similar to survival of LDT EDBs on Nemours. Multiple factors likely drive this high post-translocation survival. First, translocated EDBs came from a source population with small home ranges. Average EDB home range on Parris Island, SC is about 5 and 12 ha for males and females, respectively (Waldron et al., 2012). These home-range estimates are much smaller than estimates of other South Carolina EDBs; mainland EDBs average 85 and 29 ha home ranges for males and females, respectively. Translocated EDBs had relatively small average daily movements compared to similar LDT studies (e.g., Reinert and Rupert, 1999; Plummer and Mills 2000). Since our source population has small home ranges, their post-translocation movements were smaller, which may have mitigated the metabolic costs of exploratory behavior. In fact, while many LDT studies recommend against the practice, these same studies suggest populations with smaller movements may fare better after LDT (Plummer and Mills, 2000).

Phenology appears to be an important factor affecting survival of EDBs post LDT. While it was not statistically significant, EDBs moved 120 or more days after egress fared much better than those moved less than 30 days after egress. Similarly, eastern massasauga summer translocates fared better than autumn translocates (King et al., 2004). I was surprised that the spring 2016 cohort had significantly better body condition than the summer 2017 cohort. Despite the better body condition, the spring 2016 had lower survival than the summer 2017 cohort, suggesting I failed to include other relevant covariates in EDB survival models. I suspect that the 2017 cohort benefitted from foraging opportunities and general acclimation to the active season prior to translocation, which the spring 2016 cohort did not have.

Finally, I think the quality of the recipient site habitat contributed to high survival probabilities. Translocation studies and reviews have described the importance of habitat suitability on translocation success (Griffith et al., 1989; Germano et al., 2014). I translocated EDBs to a property where EDBs had been extirpated and has committed a large tract of land to upland pine savanna, preferred habitat for EDBs (Waldron et al., 2008). I suspect the suitability and management of the property, combined with low human activity, contributed to high survival in this study.

Translocated EDBs exhibited many of the same behaviors described in other translocation studies. Many EDBs exhibited large boli throughout the study. In fact, two snakes consumed large meals (i.e., gray squirrel or larger) during the week following translocation. I observed many snakes with meals suggesting that foraging ability does not seem to be affected by translocation. I witnessed conspecific trailing as was witnessed by Reinert and Rupert (1999). I witnessed large, aberrant movements and concentric circling. EDBs appear to ‘explore’ their new landscape, based on my radio-telemetry observations. Each translocated EDB that was alive at the onset of ingress found a suitable hibernaculum. A large fire accidentally spread through the restoration area where the EDBs were hibernating. Each EDB was located the following day in the same hibernaculum unscathed by the disturbance. Finally, and most importantly, I witnessed a lot of breeding behavior. Even the EDBs dropped off at the beginning of breeding season exhibited this behavior. Exhibiting breeding behavior so soon after translocation suggests minimal impact of LTD on breeding behavior.

The brevity of this study (i.e. 39 weeks) does not allow for a declaration of success or failure. Many papers and studies describe the need for long-term monitoring in order to make a judgement on the success of a translocation (Dodd and Seigel, 1991; Reinert, 1991). I do not disagree with the need for long-term monitoring before declaring a translocation successful. However, through this study, I have identified a factor that influences post-translocation survival of EDBs and I suspect phenology may play a role in survival of other crotalid translocations. While many papers have reviewed translocation practices and determinants of success, these same papers have described an individual approach to each species being translocated (Germano et al., 2014). Reviewers and authors alike describe differences in what influences the success of translocations among species. I suspect many other factors such as habitat integrity, habitat management process, and human activity/encroachment affect the probability of a given species surviving translocation. My survival models suggest an influence of phenology on LDT survival. I encourage other translocation studies to identify other factors that influence a given species' survival in translocation. While translocation is not a preferred method for conserving snake species, it is a method we may have no choice but to use for populations that cannot disperse on their own. Therefore, more research into the factors affecting LDT survival is needed.

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APPENDIX A

LETTER FROM IRB

MARSHALL UNIVERSITY.
www.marshall.edu

Office of Research Integrity

July 2, 2018

Mike Jungen
W7629 Autumn Court
Greenville, WI 54942

Dear Mr. Jungen:

This letter is in response to the submitted thesis abstract entitled *"Eastern Diamondback Rattlesnake Translocation at the Marine Corps Recruit Depot, Parris Island."* After assessing the abstract it has been deemed not to be human subject research and therefore exempt from oversight of the Marshall University Institutional Review Board (IRB). The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the study under protocol #640. The applicable human and animal federal regulations have set forth the criteria utilized in making this determination. If there are any changes to the abstract you provided then you would need to resubmit that information to the Office of Research Integrity for review and a determination.

I appreciate your willingness to submit the abstract for determination. Please feel free to contact the Office of Research Integrity if you have any questions regarding future protocols that may require IRB review.

Sincerely,

Bruce F. Day, ThD, CIP
Director